

Loss of SMARCA4 (BRG1) Protein Expression by Immunohistochemistry in Small Cell Carcinoma of the Ovary, Hypercalcaemic Type Distinguishes these Tumors from their Mimics.

Abstract

Aims

Molecular investigation of small-cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) has revealed that it is a monogenetic tumour characterized by alteration of *SMARCA4* (*BRG1*), encoding a member of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodelling complex. A large majority of cases show loss of expression of the corresponding SMARCA4/BRG1 protein. Furthermore, three cases of SCCOHT with retained SMARCA4 protein expression showed loss of SMARCB1/INI1 expression. The aim of this study was to assess the sensitivity and specificity of loss of SMARCA4 expression as a diagnostic test for SCCOHT.

Methods and results

We performed SMARCA4 and SMARCB1 staining in 245 tumours, many of which were potentially in the differential diagnosis of SCCOHT. We also stained 56 cases of SCCOHT for SMARCA4 and 37 of these for SMARCB1. Fifty-four of the SCCOHT cases showed complete absence of SMARCA4 expression. The two cases with retained expression showed molecular alteration of *SMARCA4*. Of the 217 other neoplasms with interpretable staining, all retained SMARCA4 expression. Although the majority showed diffuse, strong nuclear expression, a heterogeneous, typically weak staining pattern was present in 13% of cases. All 37 cases of SCCOHT tested and all other neoplasms, apart from three malignant rhabdoid tumours, showed retained nuclear SMARCB1 expression. Loss of SMARCA4 expression had a sensitivity of 96.55% and specificity of 100%.

Conclusions

Loss of SMARCA4 expression is sensitive and specific for SCCOHT. Although some mimics show heterogeneous

expression, there is retention of nuclear staining in at least a part of the tumour; therefore, only complete loss of staining should be regarded as being supportive of SCCOHT.

Introduction

Small-cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is a rare ovarian tumour that is often associated with a very poor outcome. In its original description by Scully, the key diagnostic elements were: (i) the presence of small, highly mitotic cells with hyperchromatic nuclei and scant cytoplasm; (ii) an early age (generally <40 years) of onset; and (iii) the presence of hypercalcaemia.[1] In a follow-up study of 150 cases, the mean age at diagnosis was 23.9 years, but, notably, 38% of patients did not have preoperative hypercalcaemia. Paradoxically, half of the tumours also contained large cells with abundant eosinophilic cytoplasm; when these predominate, the tumour is referred to as the 'large-cell variant' of SCCOHT.

There may be considerable morphological overlap between SCCOHT and an array of ovarian and extra-ovarian neoplasms of both the small-cell and the large-cell types, such as primary and metastatic small-cell neuroendocrine carcinoma, various sex cord–stromal tumours (including adult and juvenile granulosa cell tumour), various malignant germ-cell tumours, endometrial stromal sarcoma, primitive neuroectodermal tumour, neuroblastoma, desmoplastic small round-cell tumour, undifferentiated carcinoma, malignant lymphoma, and malignant melanoma. The diagnostic difficulties are compounded by the rarity of SCCOHT and the lack of a specific biomarker. In a detailed immunohistochemical analysis of a series of these neoplasms, most cases were found to show diffuse nuclear positivity with an antibody against the N-terminus of Wilms tumour 1; although this has some diagnostic use, this marker is positive in many other tumours, including some in the differential diagnosis of SCCOHT

Biallelic inactivation of *SMARCA4* [encoding a member of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodelling complex], either through two intra-genic (usually

exonic) mutations or a single intra-genic mutation and loss of heterozygosity (LOH) on chromosome 19p, has recently been identified as the defining molecular event in SCCOHT. These changes occur in an otherwise stable, diploid genome, and may involve the germline DNA. It has been shown that a large majority of cases also show loss of expression of the corresponding SMARCA4 protein (also referred to as BRG1). Furthermore, three cases of SCCOHT with retained SMARCA4 expression showed loss of expression of SMARCB1 (also referred to as INI1), another core member of the SWI/SNF complex; one case was found to harbour a frameshift mutation in *SMARCB1*.

Cognition between SCCOHT, atypical teratoid/rhabdoid tumour of the brain and malignant rhabdoid tumour of the kidney is suggested by their shared genomics, with all three tumours having mutations in SWI/SNF genes (either *SMARCA4* or *SMARCB1*) in otherwise stable genomes. This molecular insight has been epiphanic, revealing their now seemingly obvious morphological and clinical similarities (young patients, highly aggressive tumours, variable occurrence of hypercalcaemia, and occasional hereditary transmission). This has led to the proposal that SCCOHT represents a malignant rhabdoid tumour of the ovary.

In the initial articles describing the molecular alterations and in subsequent follow-up studies, loss of SMARCA4, or rarely SMARCB1, expression has been shown to be highly sensitive for SCCOHT, being demonstrated in 109 of 114 (96%, including 106 SMARCA4-deficient and three SMARCB1-deficient) cases (Table 1) (Figure 1). To date, ~3600 cases of ovarian (epithelial, sex cord–stromal, and germ-cell), uterine (epithelial and stromal) and non-gynaecological tumours have been studied (Tables 2 and 3) for SMARCA4 expression, with the vast majority of cases showing retained expression. This suggests that SMARCA4 is a highly sensitive and specific marker for SCCOHT. However, as a broad array of primary and considered in the differential diagnosis, it is important to focus on the various potential mimics. The previous

immunohistochemical studies have included limited numbers of some of the less common mimics of SCCOHT. We therefore aimed to extend prior observations by analysing SMARCA4 expression in an additional cohort of entities, many of which are rare but often considered in the differential diagnosis of SCCOHT. We also stained additional cases of SCCOHT for SMARCA4, and analysed SMARCB1 expression in a subset of the SCCOHT cohort.

Table 1. Summary of previous and current studies examining the immunohistochemical expression of SMARCA4 in cases of small-cell carcinoma of the ovary, hypercalcaemic type

Figure 1.

A, Small-cell carcinoma of the ovary, hypercalcaemic type; haematoxylin and eosin. **B,** Loss of SMARCA4 expression in small-cell carcinoma of the ovary, hypercalcaemic type. Note retained expression in endothelial cells and lymphocytes.

Table 2. SMARCA4 expression in ovarian tumours published previously

Table 3. SMARCA4 expression in uterine epithelial and mesenchymal tumours and non-gynaecological tumours published previously

Materials and methods

Case selection and tissue microarray (TMA) construction

Through a multicentre collaboration involving the institutions to which the authors are affiliated, 245 potential morphological mimics of SCCOHT were collected, together with a range of ovarian sex cord–stromal tumours. For the majority of cases, TMAs were constructed with duplicate 0.6-mm cores. For those cases in which only unstained sections could be obtained, whole sections were stained. Included in the survey were previously constructed single-core TMAs of neuroblastomas (13 cases), Wilms tumours (18 cases), and peripheral neuroectodermal tumours (12 cases). Table 4 shows the tumour subtypes included in the study cohort.

Table 4. SMARCA4 expression in tumours other than small-cell

carcinoma of the ovary, hypercalcaemic type examined in the current study

As a control group, we included 56 cases of SCCOHT, 41 of which have been published previously (including both genetic and immunohistochemical results).[9, 16] All 56 cases were stained with SMARCA4/BRG1, and 37 of these cases, which were included in a TMA of SCCOHT, were also stained for SMARCB1/INI1.

Immunohistochemical staining and interpretation

Four-micrometre sections of TMAs and whole sections were processed with the Ventana Discovery Ultra system (Ventana Medical Systems, Oro Valley, AZ, USA), with a rabbit monoclonal antibody against SMARCA4 (BRG1; ab110641; 1:25 dilution; Abcam, Cambridge, UK). Cases were stained with monoclonal anti-BAF47 (INI1/SMARCB1) antibody (clone 25; BD; 1:50 dilution; Transduction Laboratories, San Diego, CA, USA). All cases were stained with SMARCA4, and SMARCB1 staining was performed on all non-SCCOHT cases and on a subset of the SCCOHT which include those rare cases with retained SMARCA4 expression. The arrays and whole sections were scored by two pathologists (B.A.C. and W.G.M.). Any nuclear expression of SMARCA4 or SMARCB1 in tumour cells was regarded as positive. For a case to be regarded as negative (to show loss of staining), there had to be complete absence of nuclear staining in tumour cells, together with positive nuclear staining of an internal positive control (endothelial cells, fibroblasts, or lymphocytes). Cases showing absence of staining in both the tumour and the internal controls were regarded as uninterpretable.

Results

Table 4 summarizes the results of the non-SCCOHT cases. For SMARCA4, 217 cases (88.6%) could be interpreted, and all showed retained/intact expression. Case attrition was predominantly attributable to core drop-out or lack of tumour representation, with only one case showing absence of staining in internal control cells. One hundred and eighty-nine (87.1%) tumours showed diffuse moderate to strong nuclear staining

with SMARCA4 (Figure 2), whereas 28 (13%) showed heterogeneous nuclear staining, usually involving <30% of tumour cells; in the majority of the latter cases, staining was weak to moderate, with a single tumour showing strong but focal staining (Figure 3). Those cases showing heterogeneous weak/moderate expression comprised one immature teratoma, one mixed germ-cell tumour with predominant choriocarcinoma differentiation, three steroid cell tumours, three unclassifiable sex cord–stromal tumours, two adult-type granulosa cell tumours, one malignant melanoma metastatic to the ovary, one lymphoma involving the ovary, one desmoplastic small round-cell tumour, nine fibromas/fibrothecomas, and five gonads from patients with androgen insensitivity syndrome. The single case showing strong focal staining was an unclassified sex–cord stromal tumour. SMARCB1 staining was intact in all non-SCCOHT cases, apart from the three malignant rhabdoid tumours.

Fifty-four of the 56 SCCOHT cases showed complete loss of SMARCA4 expression (Figure 1). All 37 of the SCCOHT cases stained with SMARCB1 showed intact nuclear expression. These included the two cases with retained SMARCA4 staining. The latter two cases did show molecular alteration of *SMARCA4*, and morphologically resembled the other SCCOHT cases. In the cohort examined, loss of SMARCA4 expression had a sensitivity of 96.55% and a specificity of 100%.

Figure 2.

A, Ovarian dysgerminoma; haematoxylin and eosin (H&E). **B**, Strong, diffuse nuclear SMARCA4 expression in dysgerminoma. **C**, Burkitt's lymphoma involving the ovary; H&E. **D**, Strong, diffuse nuclear SMARCA4 expression in Burkitt's lymphoma.

Figure 3.

A, Unclassified sex cord–stromal tumour; haematoxylin and eosin (H&E). **B**, Unclassified sex cord stromal tumour showing strong but heterogeneous SMARCA4 expression. **C**, Adult-type granulosa cell tumour; H&E. **D**, Adult-type granulosa cell tumour showing weak heterogeneous SMARCA4 expression.

Discussion

Inactivation of a member of the SWI/SNF chromatin remodelling complex, *SMARCA4*, has recently been identified as the defining molecular event in SCCOHT.[6-9] This complex modulates nucleosome structure, thereby regulating DNA–protein interactions in processes such as transcription, replication, and repair.[23, 24] *SMARCA4* mutations identified in SCCOHT are typically nonsense, small indels leading to frameshifts, or splice-site mutations, and rarely missense mutations.[6, 8, 9] These coding sequence mutations are often accompanied by LOH of the wild-type allele, and occur in a stable, diploid genome; in a subset of cases (up to one half in some studies), mutations affect the germline DNA.[6-9] Rare cases of SCCOHT lacking molecular and immunohistological evidence of *SMARCA4* deficiency show alterations in *SMARCB1*, representing a mechanism of oncogenesis via alternative defects in the SWI/SNF complex. Of three such cases (retained *SMARCA4* expression/loss of *SMARCB1* expression) described to date, two in which *SMARCA4* was investigated lacked molecular alteration of this gene; one case showed a frameshift mutation in *SMARCB1*, whereas the other was wild type upon genetic analysis.[11] Overall, 96.1% (124/129) of SCCOHT cases have been found to show loss of expression of either *SMARCA4* (121 cases) or *SMARCB1* (three cases).

SCCOHT may rarely show retained *SMARCA4* staining

There are five reported cases of purported SCCOHT with retained or equivocal *SMARCA4* expression, and these are summarized in Table 5. In four of these cases, a mutation was identified in *SMARCA4*, confirming the diagnosis despite the retained protein expression. Two of these cases were in the previous publication by Witkowski *et al.*[9] One tumour showed weak staining with *SMARCA4*, and harboured a monoallelic splice-site mutation in *SMARCA4*, predicted to result in an exonic in-frame deletion, and there was no LOH on chromosome 19p. The second case was from a patient with a germline missense mutation, and the tumour also showed

somatic LOH; interestingly, her daughter, with the same germline mutation, had an SCCOHT with a somatic frameshift mutation, but this showed loss of SMARCA4 staining. These resembled other SCCOHT cases histologically. In the study by Jelinic *et al.*, a splice-site mutation in *SMARCA4* was identified in the case with equivocal protein expression.[6] In the other SCCOHT case with intact SMARCA4 expression in that study, the tumour harboured a homozygous in-frame deletion affecting amino acids of the helicase domain, postulated to result in a truncated inactive protein. The fifth case lacked molecular alterations in either *SMARCA4* or *SMARCB1*, and showed retained expression of both proteins. These findings raise the possibility of misdiagnosis, but could also indicate a novel mechanism of oncogenesis in SCCOHT.[8]

Table 5. Summary of reported small-cell carcinoma of the ovary, hypercalcaemic type cases with retained/equivocal SMARCA4 expression

In light of these rare cases, if a morphological diagnosis of SCCOHT is favoured, and both SMARCA4 expression and SMARCB1 expression are intact, molecular testing of *SMARCA4* may be indicated. However, given the rarity of this finding, when there is retained immunohistochemical expression of SMARCA4, other tumours in the differential diagnosis should be strongly considered.

In ovarian neoplasms, loss of SMARCA4 expression is contextually specific to SCCOHT

SCCOHT is the prototypical ovarian neoplasm composed predominantly or exclusively of small round cells with scant cytoplasm (so-called 'small round blue cell tumour'). The differential diagnosis of such neoplasms is wide, and pathologists commonly struggle with these cases, owing to their overlapping morphological and immunohistochemical features.[3, 5] Many of these tumours occur in young women, are highly aggressive, and require specific chemotherapeutic agents, making a correct diagnosis imperative. In diagnosing the various tumour types, immunohistochemistry, as well as molecular studies, may be of value. Although typical SCCOHT

is part of the differential diagnosis of a small round blue cell tumour, the large-cell variant of SCCOHT may be confused with a variety of neoplasms composed of large cells, such as undifferentiated carcinoma, large-cell neuroendocrine carcinoma, and malignant melanoma. The results of the current and prior studies indicate that loss of SMARCA4 expression is a highly sensitive and specific immunohistochemical marker of SCCOHT. Of the ~3600 cases of non-SCCOHT ovarian, uterine and non-gynaecological tumours studied to date (Tables 2 and 3), only a few cases have been noted to lack expression of SMARCA4: one case of uterine endometrioid carcinoma (1/360; <1%); 16 cases of endometrial dedifferentiated/undifferentiated carcinoma (16/59; 27%); four cases of uterine endometrial stromal sarcoma (4/53; 7.5%); one melanoma metastatic to ovary of the 47 primary or metastatic melanomas tested (1/47; 2.1%); and 17 ovarian clear-cell carcinomas (17/447; 3.8%), although these tumours are not typically considered in the differential diagnosis of SCCOHT.[8-10, 14, 17-22]

Although a single case of uterine endometrioid carcinoma was found to lack SMARCA4 expression, this has not been demonstrated in any of the 343 cases of ovarian endometrioid carcinoma examined.[9, 10, 17]

Only a single case of primary ovarian endometrial stromal sarcoma has been studied, and it was found to retain SMARCA4 expression, but further work is warranted, as 7.5% of uterine endometrial stromal sarcomas show loss of SMARCA4 expression.[10] Finally, 16 of 59 (27%) endometrial dedifferentiated/undifferentiated carcinomas showed loss of SMARCA4 expression, but, of the 22 cases of ovarian undifferentiated/dedifferentiated carcinoma examined to date, only one has shown loss of SMARCA4 expression, and in this case it was noted in the undifferentiated component.[9, 10, 22]

Another useful marker for SCCOHT is SMARCA2, a mutually exclusive SWI/SNF ATPase. Karnezis *et al.* recently demonstrated dual loss of SMARCA4 and SMARCA2 expression in 42 SCCOHT cases, and dual loss of SMARCB1

and SMARCA2 expression in two cases.[10] None of the ovarian clear-cell carcinomas showed dual loss of expression, suggesting this is specific for SCCOHT among primary ovarian tumours. In uterine tumours, however, dual loss of expression was noted in one case of dedifferentiated carcinoma and in two cases of high-grade endometrial stromal sarcoma.[10] In a similar study, Jelinic *et al.* showed dual loss of SMARCA4 and SMARCA2 expression in nine of 10 SCCOHT cases. Of the other 50 tumours stained (20 ovarian clear-cell carcinomas, 10 ovarian granulosa cell tumours, 10 metastatic pulmonary small-cell carcinomas, and 10 metastatic melanomas involving the ovary), none showed loss of expression of both markers, although loss of SMARCA4 expression was seen in one clear-cell carcinoma and in one metastatic melanoma.[18]

In our study, we included a large number of rare tumours that could present as morphological mimics of SCCOHT, including a wide array of small round blue cell tumours. Although some of these neoplasms have been included in prior studies, the number of cases examined has been relatively limited. Our data confirm that loss of SMARCA4 expression is highly sensitive and specific for SCCOHT. We demonstrate that a broad spectrum of tumours (Table 4), many of which are commonly considered in the differential diagnosis of SCCOHT, show intact SMARCA4 expression. Furthermore, in our study, SMARCB1 staining was also intact, except in cases of malignant rhabdoid tumour.

Diagnosis of SCCOHT by SMARCA4 staining requires complete absence of staining and positive internal controls

Although the majority of potential histological mimics showed diffuse, strong SMARCA4 expression, 28 (13%) cases showed focal expression in <30% of tumour cells. These cases generally showed weak-intensity to moderate-intensity staining, with a single case showing strong staining. This heterogeneous pattern of staining involved a number of tumour subtypes, and may be attributable to fixation issues in individual cases.

However, the over-representation of certain tumour types, such

as 50% (9/18) of fibromas/fibrothecomas, suggests that this is an intrinsic property of these particular neoplasms. Although fibromas/fibrothecomas do not enter into the differential diagnosis of SCCOHT, unclassified sex cord–stromal tumours may be considered, and 31% (4/13) of these neoplasms showed this heterogeneous pattern of staining. We acknowledge that TMAs may constitute a possible confounding factor, but, in the immunohistochemical study performed on full sections by Karanian-Philippe *et al.*, a similar pattern of heterogeneity was noted in adult-type granulosa cell tumour (46% of 44 cases), desmoplastic small round-cell tumour (77% of nine cases), and Ewing's sarcoma (24% of 13 cases).[14] Thus, although loss of SMARCA4 expression is highly sensitive and specific for SCCOHT, accurate diagnosis requires a complete absence of staining in tumour cells with intact positive internal controls. The possible heterogeneity and the variable intensity of staining warrants caution in the interpretation of SMARCA4 staining, especially in small biopsy specimens.

Conclusion

Accurate histological classification of ovarian tumours is critical in ensuring appropriate patient management. Rare tumours such as SCCOHT may present diagnostic difficulty, owing to their rarity and, hence, unfamiliarity to many diagnostic pathologists, and their morphological overlap with other neoplasms. The current study confirms that loss of SMARCA4 expression by immunohistochemistry is a sensitive and specific marker for SCCOHT, and, in the vast majority of cases, should resolve any diagnostic dilemmas. However, a number of caveats must be borne in mind. Accurate diagnosis of SCCOHT requires a complete absence of staining of tumour cells with an intact positive internal control, as some morphological mimics can show heterogeneous weak staining. This is most pertinent in biopsy samples. A single case of metastatic melanoma (1/47, 2.1%) involving the ovary has been demonstrated to show loss of SMARCA4 expression, and, in such cases, retained staining of SMARCA2 may be used to exclude SCCOHT.[17, 18]

Furthermore, on the basis of the current data, if the morphological suspicion of SCCOHT is high and SMARCA4 expression is intact, immunohistochemical testing for SMARCB1 should be performed. In the event of retained expression of both markers, an alternative diagnosis should be strongly considered, and an expanded panel of immunomarkers should be used to exclude entities in the differential diagnosis. As mutation-positive cases may rarely show retained protein expression, molecular testing may be required as the final arbiter.

Conflict of interest

The authors state that they have no conflicts of interest.